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Cross-kingdom comparison of the developmental hourglass

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The developmental hourglass model has its foundations in classic anatomical studies by von Baer and Haeckel. In this context, even the conservation of animal body plans has been explained by evolutionary constraints acting on mid-embryogenic development. Recent studies have shown that developmental hourglass patterns also exist on the transcriptomic level, mirroring the corresponding morphological patterns. The identification of similar patterns in embryonic, post-embryonic, and life cycle spanning transcriptomes in plant and fungus development, however, contradict the notion of a direct coupling between morphological and molecular patterns. To explain the existence of hourglass patterns across kingdoms and developmental processes, we propose the organizational checkpoint model that integrates the developmental hourglass model into a framework of transcriptome switches.

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Introduction

Understanding genesis, evolution, and variability of complex organismal forms is among the most fundamental objectives of biological research. *Embryogenesis* (Box 1) is the key process to establish complex multicellular life in animals and plants by transitioning from a single-celled zygote to a mature multicellular embryo [1]. The organizational capacity of embryogenesis provides the developmental framework to establish the *body plan* (Box 1) of a multicellular organism. This usually happens by passing through a defined series of developmental stages governed by specific programs of gene expression [1].

A central question arising from the body plan concept is why and to what extent the basic body plan is conserved within and between phyla [2–4]. One prominent model to address these questions is derived from the observation that animal embryos of different species within a phylum converge to a form of considerable morphological resemblance during the organogenic period in mid embryogenesis, while appearing rather dissimilar in early and late embryonic stages. This mid-developmental window where embryos of different species are morphologically similar has been termed *phylotypic stage* [5] or *phylotypic period* [6–8] (Box 1). The morphological pattern in general has been described as the *developmental hourglass model* (Figure 1a and Box 1), which assumes that *developmental constraints* (Box 1) maximize during mid embryogenesis [6,9], resulting in morphological conservation in this phase. Today, based on the developmental hourglass model conserved stages during embryogenesis and their role in constraining the animal body plan are investigated. In a broader context, this model is used to speculate about the origin and conservation of extant animal body plans. It connects body plan emergence during the Cambrian Explosion with constraints acting on mid-embryonic development [4].

Historically, several hypotheses have been proposed as possible explanations for the morphological resemblance during the phylotypic period. Applying the terminology of Schleip [10], Sander [5] hypothesizes that the transition from *primitive development* (Box 1) to *definitive development* (Box 1) marks a crucial phase during embryogenesis. In this notion, the similarity between organismal forms is caused by developmental constraints that conserve this transition. Other authors such as Duboule [6] suggested that in vertebrates the conserved sequential activation of *HOX* patterning genes causes the apparent invariance of embryos during the phylotypic period. Raff [9] hypothesizes that a high interconnectivity of complex interactions between discrete modules (*e.g.* organ primordia) constrains mid-embryonic development. These largely accepted explanations suggest that (i)

Box 1 Explanation of specific terms.

Embryogenesis

Embryogenesis is the key process to establish complex multicellular life by transitioning from a single-celled fertilized egg (= zygote) to a mature multicellular organism (= embryo). As the last common ancestor was most likely unicellular, embryogenesis evolved independently in animal and plant kingdoms. Each kingdom generates different degrees of multicellular complexity. In animal embryogenesis ontogenetic development is largely completed by the end of embryogenesis, whereas plant embryos are less complex and the majority of organs form during post-embryonic development.

Body plan

The body plan (initially termed 'Bauplan') characterizes the morphological features that are shared between species within a phylum. The body plan of mature embryos in most animal lineages is practically identical to adult individuals. Typical body plan features for animals are head, limbs, fins, and so on. In plants, mature embryos have only established the basic body plan. Their ability for post-embryonic organogenesis results in various adaptations of the body plan during a plant's life cycle, often in response to environmental stimuli. Typical body plan features for plants are, for example, roots, stem(s), leaves, and flowers.

Phylotypic stage/period and developmental hourglass model

Comparative embryology studies performed in the past two centuries revealed that mid stage embryos of different animal species within the same phylum converge to a form of high morphological resemblance when compared with early and late embryogenesis. Because of the high morphological resemblance of anatomical features shared between different vertebrate taxa, this developmental window has been termed phylotypic stage or phylotypic period. The morphological pattern of dissimilarity – similarity – dissimilarity between animal embryos has been termed developmental hourglass. It has been argued that the phylotypic period is connected with the establishment of body plans. In particular the developmental hourglass model postulates that strong developmental constraints during the phylotypic period are causing the limited diversification of animal body plan features during evolution.

Developmental constraints

A limitation on phenotypic variability caused by the structure, character, composition, or dynamics of the developmental system which, thus, limits the potential combinatorial variability of morphologies.

Primitive vs. definitive development

The classical embryologist Waldemar Schleip categorized animal embryogenesis in two major phases: primitive development defining the early part of embryogenesis that is characterized by cell differentiation and definitive development denoting the period of embryogenesis where development is mainly characterized by cell growth.

Comparative and phylotranscriptomics

While comparative transcriptomic approaches measure expression divergence of orthologous genes in two or more related species, the term phylotranscriptomics summarizes a collection of procedures and methods that aim to quantify the evolutionary age or evolutionary conservation of transcriptomes. For this purpose, gene age or protein evolutionary divergence rate information is combined with expression levels covering a biological process of interest, usually in a single species. Hence, phylotranscriptomics allows to retrieve the average transcriptome age or transcriptome divergence for each stage of a biological process.

Meristem

Undifferentiated totipotent stem cells that form plant tissues. The plant body plan consists of two primary meristems: the shoot apical meristem (SAM) that gives rise to the shoot of the plant and the root apical meristem (RAM) that gives rise to the root of the plant. At least during vegetative development, apical meristems are of an indeterminate nature, which underlies the plants' ability to adapt their body plans throughout almost the complete life cycle.

Phytomer

Functional units of plants produced by the apical meristems. A phytomer unit consists of a leaf which is attached to a node, an axillary bud at the base of a leaf, and an internode as the stem section between two nodes. Phytomers denote the smallest unit of vegetative modules.

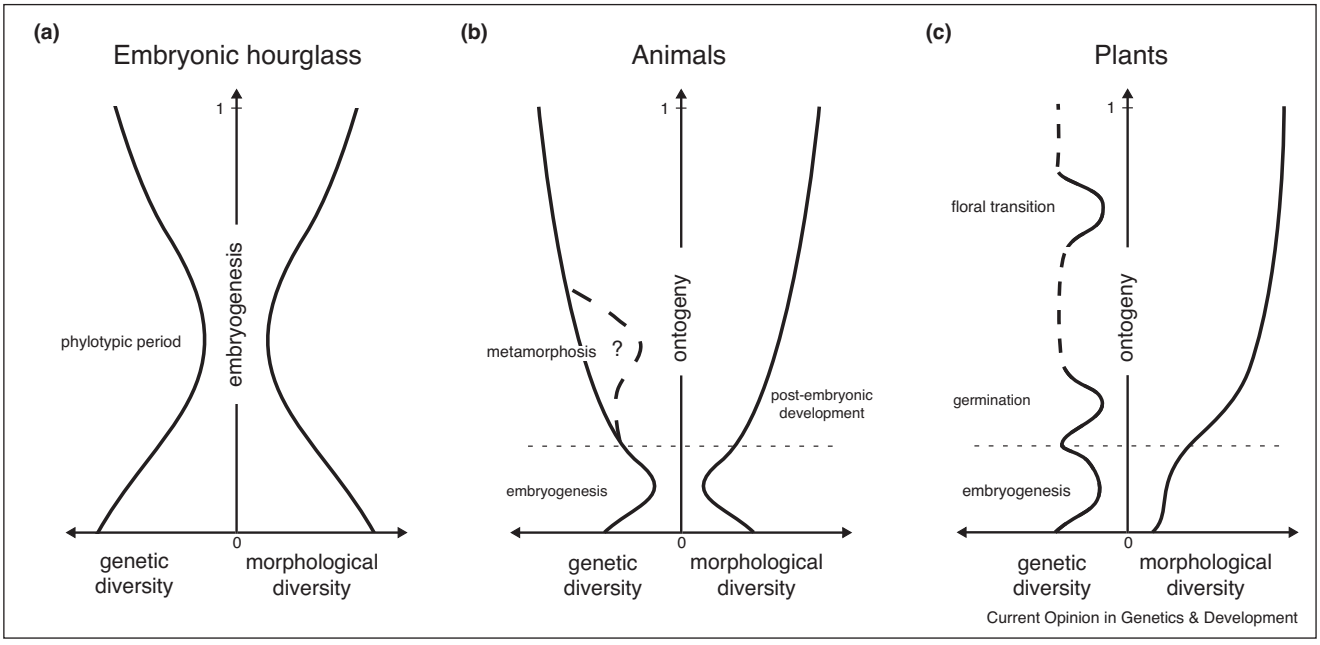
the developmental hourglass model is restricted to embryogenesis and (ii) constraints on organogenesis or body plan formation in mid embryogenesis are believed to be the main cause for the morphological resemblance of animal embryos in that stage or period.

In this review, we take a comparative perspective on the developmental hourglass model. We review recent molecular findings in animals and plants, discuss their (in)compatibility with the developmental hourglass model originally conceived to explain morphological hourglass patterns in animals, and propose the organizational checkpoint model that is compatible with those findings in both animals and plants.

Is there a cause-and-effect relation between transcriptomic and morphological hourglass patterns?

Although the existence of a phylotypic stage or period has been controversially debated and some studies have questioned the validity of the developmental hourglass model [11–14], a recent wave of gene expression studies has largely supported it on the molecular level (reviewed in Ref. [4]). Two different transcriptomic approaches

Figure 1

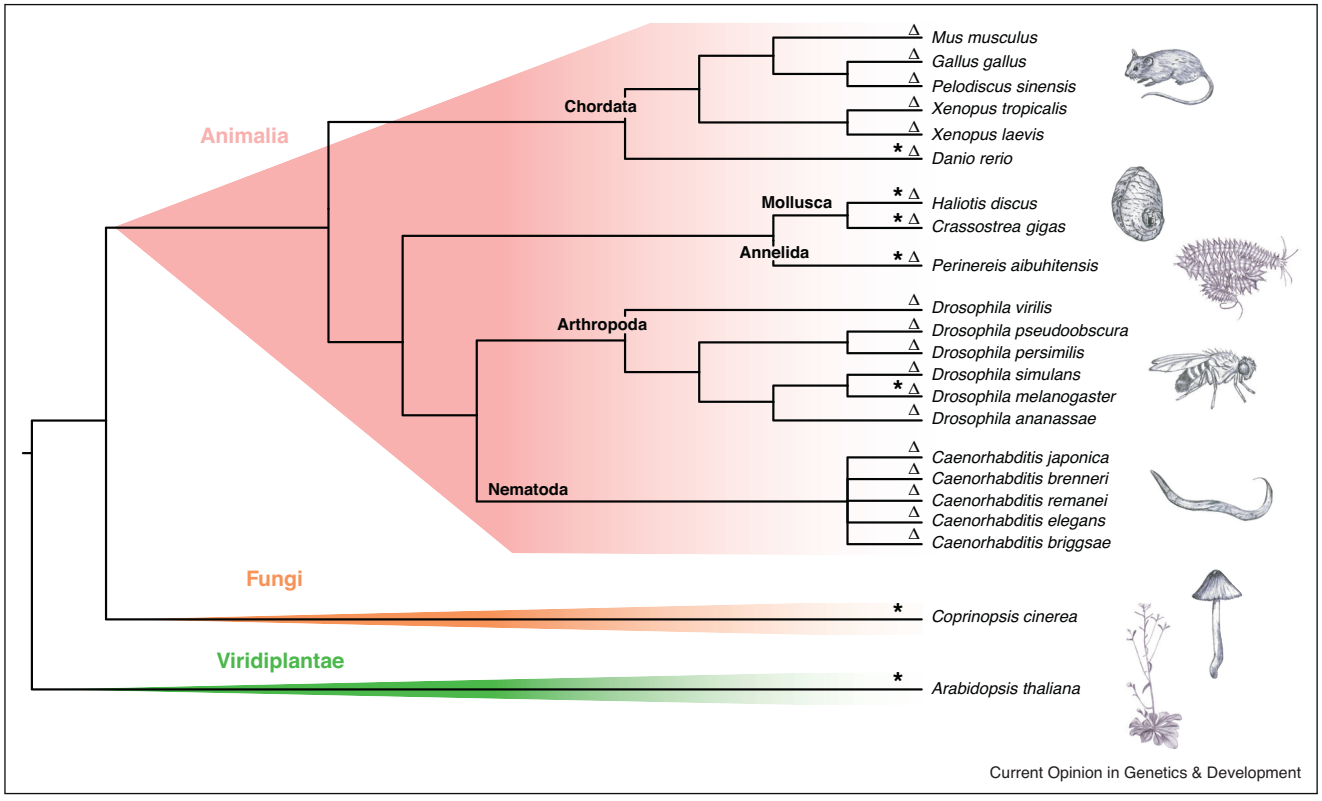


Comparing the molecular and morphological developmental hourglass model in plants and animals. **(a)** The classical developmental hourglass model in animal embryogenesis. The model predicts a stage of maximum conservation (phylotypic period) between species from the same phylum on both the morphological and the genetic level in mid embryogenesis. **(b)** Extending the model to a complete animal life cycle suggests that a period of morphological and genetic conservation is likely specific for mid embryogenesis and probably lacking from post-embryonic development. However, we hypothesize that another conserved stage can exist for species passing through metamorphosis. **(c)** For plants, so far no conclusive information has been reported for morphologically conserved stages throughout the life cycle. On the transcriptomic level several highly conserved stages can be found for various embryonic and post-embryonic transitions (germination and floral transition), suggesting that at least in plants the hourglass phenomenon is not restricted to embryogenesis. Dashed lines illustrate unexamined periods (metamorphosis in animals and vegetative growth and fruit development in plants). (a–c) Although accumulated into schematic illustrations for all animals and plants, morphological hourglass patterns are restricted to single phyla within each corresponding kingdom.

have been used to quantify the similarity of developing embryos. In the first approach, *comparative transcriptomics* (Box 1), the conservation of gene expression patterns of orthologous genes is quantified (reviewed in Refs. [15,16]), while in the second approach, *phylotranscriptomics* (Box 1), the evolutionary age of developmental transcriptomes of a single species is quantified [17]. The latter approach does, therefore, not evaluate whether or not the same orthologous genes are expressed in embryos of different species. In theory, it is possible that the developmental stage with the highest transcriptome conservation as measured by phylotranscriptomics and the stage with the highest expression similarity of orthologous genes as measured by comparative transcriptomics are not identical. Despite their different methodologies, both approaches independently demonstrated that the conservation of expression patterns of orthologous genes or the mean evolutionary age of expressed genes, respectively, are maximal during mid embryogenesis in fly, zebrafish, and worm (shown by both phylotranscriptomics and comparative transcriptomics) as well as frog, mouse, chicken, turtle, oyster, sandworm, abalone, and/or various additional animal species from different phyla (shown by comparative transcriptomics) [17–24,25••,26] (Figure 2). Interestingly, these conservation patterns on the transcriptomic level often, but not always, mirror conservation patterns on the morphological level [25••,26].

The finding of developmental hourglass patterns on the transcriptomic level raised the legitimate expectation that these patterns would help us understand the morphological pattern and thus the establishment of body plans. One could argue, however, that the finding of developmental hourglass patterns on the transcriptomic level is caused by the current *zeitgeist* that likely favours studies that successfully identify transcriptomic hourglass patterns. In contrast to this 'self-fulfilling prophecy' it is unknown how many studies have failed to identify such patterns and therefore remained unpublished. On the other hand, while we have recently experienced a flood of transcriptomic hourglass patterns, a detailed analysis of the matching of transcriptomic and morphological hourglass patterns has been largely neglected. To our knowledge, the seminal comparative embryology study in vertebrates by Richardson *et al.* [8], which aimed to assess the validity of the morphological hourglass model (i) to date remains the

Figure 2



On the transcriptomic level, developmental hourglass patterns have been described in all eukaryotic kingdoms. The cladogram is showing the evolutionary relationships of these species. Asterisks denote studies based on phylotranscriptomics. Triangles specify studies based on comparative transcriptomics. Monophyletic groups of the cladogram are colour coded: animalia (red), fungi (orange), and viridiplantae (green). Phyla are specified in the branches and leaves of the cladogram represent the species.

only in depth morphological study that rigorously tested morphological transitions throughout embryogenesis and across species, (ii) includes only a fraction of the species for which transcriptomic hourglass patterns have recently been found, and (iii) was not especially supportive of a simplified hourglass model. This notion is reflected by the introductory statement given by Richardson *et al.* [8]: "*One puzzling feature of the debate in this field is that while many authors have written of a conserved embryonic stage, no one has cited any comparative data in support of the idea. It is almost as though the phylotypic stage is regarded as a biological concept for which no proof is needed.*" Hence, in contrast to quantifyable and even statistically testable transcriptome studies, a convincing quantification of morphological similarities between species remains a challenge despite the use of common morphological markers.

A causal relationship between transcriptomic and morphological patterns has, therefore, yet to be demonstrated. To demonstrate or reject that relationship requires studies that (i) demonstrate or reject that major changes on the transcriptome conservation level lead to major changes on the morphological level or vice versa and/or (ii) display transcriptomic and/or morphological hourglass patterns for developmental processes unrelated to embryogenesis and body plan establishment. If hourglass patterns are not restricted to embryogenesis, it would be interesting to ascertain whether such patterns could be explained by mechanisms similar to the above described Sander/Duboule/Raff hypotheses.

Is there a developmental hourglass pattern in plant embryogenesis?

In plants, very little is known about the existence of morphologically conserved stages during embryogenesis and their correlation with the conservation of body plans. Some morphological studies suggest that mid-stage embryos of dicots are morphologically conserved [27–29], but convincing evidence is missing. Furthermore, embryos of dicots and monocots, the two major lineages within the flowering plants, differ dramatically on the morphological level ([29,30••,31,32], reviewed in Ref. [30••]). Assumptions about a possible existence of morphological hourglass patterns in plant embryogenesis are, therefore, at best inconclusive.

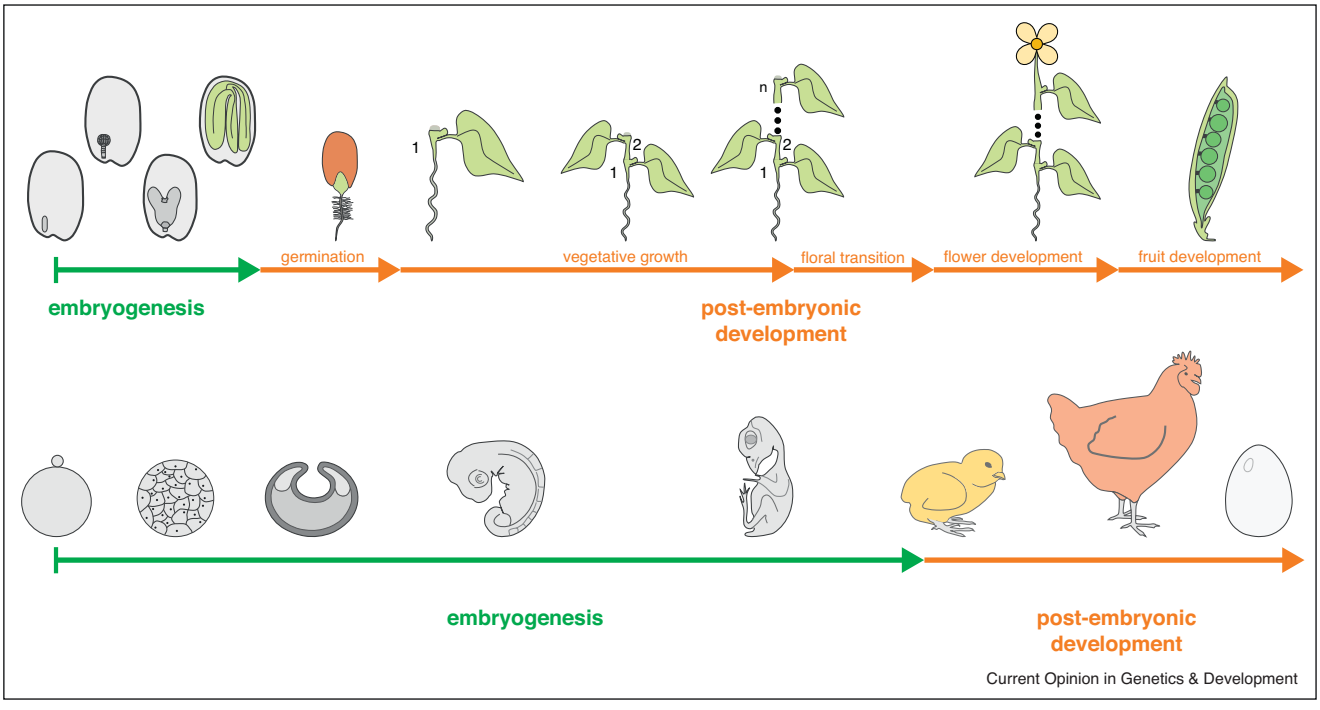
Molecular approaches have shed new light on this subject also in plants. Specifically, a significant hourglass pattern has been found by phylotranscriptomic analyses of *Arabidopsis thaliana* embryogenesis [24,33]. Here, the most conserved transcriptomes were identified at the transition from morphogenesis to growth. Although studied in only a single plant species so far [24,33,34], the presence of a transcriptomic hourglass pattern in plants suggests that it has evolved convergently and is not an exclusive invention of the animal kingdom. A transcriptomic hourglass pattern outside the animal lineage has also been found in fungus development [35•]. As fungi do not perform embryogenesis, this pattern represented the first non-embryonic hourglass pattern and could be described as a developmental hourglass in the widest sense. In fact, this type of life cycle hourglass was comparable to the life cycle pattern in zebrafish [17].

Provided that animal and plant/fungus hourglass patterns serve a common function, the lack of solid evidence for morphological hourglass patterns in the latter two questions the putatively causal relationship between morphological and transcriptomic hourglass patterns. In fact, these findings favour a scenario in which transcriptomic and morphological patterns are uncoupled. We speculate that while the molecular level is decisive, whether or not this pattern penetrates to the morphological level (as in embryogenesis of several animal phyla) or not (as in plants and fungi, and possibly several animal phyla) is likely irrelevant.

Post-embryonic hourglass patterns in plant development lead to the organizational checkpoint model

With the exception of metamorphosis in selected lineages, animal development largely occurs during embryogenesis (Figure 3, [1]), stating that mature animal embryos have 'practically' completed ontogenetic development. Mature plant embryos, however, are far from having completed ontogenetic development (Figure 3). During embryogenesis of most dicot species, for example, the *meristems* (Box 1) give only rise to the embryonic stem (hypocotyl), the seed leaves (cotyledons), and the radicle (embryonic root). The vast majority of organs such as the root system, true leaves, stem(s), flowers, and so on are established during post-embryonic development [36]. Furthermore, in contrast to animals, organ development does not occur simultaneously but rather sequentially and

Figure 3



An ontogenetic comparison of life cycles of animals and plants. The life cycle of plants and animals are shown on an ontogenetic scale from perception to reproduction and illustrated with schematic drawings. Although not necessarily drawn on scale, ontogenetic development differs dramatically between plants and animals. In most animals development is largely embryonic. The vast majority of organs are practically simultaneously initiated during the phylotypic period and body plan complexity of mature embryos is comparable to adults. In contrast, in plants most organs develop post-embryonically. Mature embryos therefore only possess a basic body plan and are, consequently, less complex compared to adult plants. Post-embryonic plant development occurs (also) in response to environmental stimuli by sequential addition of phytomer units (Box 1).

in a modular manner as so-called *phytomer* units (Box 1, Figure 3). This makes plants – in contrast to animals – a promising model system to study the possible existence of non-embryonic hourglass patterns. We hypothesize that developmental hourglass patterns found in animal embryogenesis represent 'only' specific cases of a wide variety of yet to be discovered hourglass patterns (Figure 1b).

And indeed, transcriptomic hourglass patterns have recently been found for two additional major developmental transitions in the life cycle of *A. thaliana* [37••] (Figure 1c): the embryo-to-vegetative transition (seed germination) and the vegetative-to-reproductive transition (floral transition). In both cases the stages of maximum transcriptome conservation marked the transition point at the junction between two major developmental phases. Hence, even in the absence of organogenesis as in germination and floral transition, developmental processes are apparently channelled towards an organizational checkpoint that separates two major sequential developmental programs. It seems that this checkpoint must be passed at or right after the end of a developmental phase before a successful transition to the next developmental phase is possible. In plant and animal embryogenesis the checkpoint marks the mid-developmental transition [25••,33], in seed germination the transition from dormancy to growth [37••,38,39], and in floral transition the transition from a vegetative to a reproductive meristem [37••]. In all cases, it was shown that different sets of genes contribute to the developmental transcriptome before and after the organizational checkpoint [25••,33,37••,38,39].

Obviously, the described findings from plants and fungi suggest that transcriptomic hourglass patterns are not restricted to embryogenesis. Transcriptomic experiments in appropriate animal models might likewise identify post-embryonic hourglass patterns (*e.g.* metamorphosis, Figure 1b). Transferring the findings from plant and fungus systems to the classic developmental hourglass model, we question that developmental constraints acting on organogenesis are underlying the animal hourglass patterns. Instead, we propose the *organizational checkpoint* model to integrate the developmental hourglass model into a framework of transcriptome switches. Organizational checkpoints seem to mark stages that show maximum conservation of expression patterns. In this regard, the evolutionary conservation of checkpoints might prepare transcriptomes to switch to the next major developmental program. They thereby prohibit a larger period of overlapping of different developmental programs, ensuring ordered transition between programs.

Conclusions

Before concluding, we have to note that the unifying hypotheses proposed in this review are based on the assumption that the recently described animal and plant hourglass patterns serve a similar function in both lineages. As the function of the developmental hourglass is a matter of ongoing debate and research, this remains to be seen. On this basis, we hypothesize that transcriptomic hourglass patterns are signatures of a developmental series that is channelled to pass through an organizational checkpoint allowing the transcriptomes of developing organisms to transition from one developmental phase to another. As such, the organizational checkpoint model might explain and integrate the embryonic and post-embryonic, organogenic and non-organogenic hourglass patterns across kingdoms. In a more general context, transcriptomic hourglass patterns need not to be restricted to developmental transitions. In fact, they might reflect any channelled series of biological processes that allows organisms to switch from one (regulatory) transcriptome state to another. We anticipate that adaptation of the ideas presented here will help us to explore new directions to investigate the constraints on transcriptomic and morphological diversification, which will potentially complement the tremendous efforts already taken with regard to animal embryogenesis.

Conflict of interest statement

The authors declare no conflicting interests.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Wolpert L, Tickle C, Arias A: *Principles of Development*. Oxford University Press; 2011.
2. Meyer AW: **Some historical aspects of the recapitulation idea**. *Q Rev Biol* 1935, **10**:379-396.
3. Willmore KE: **The body plan concept and its centrality in evo-devo**. *Evol Educ Outreach* 2012, **5**:219-230.
4. Irie N, Kuratani S: **The developmental hourglass model: a predictor of the basic body plan?** *Development* 2014, **141**:4649-4655.
5. Sander K: *The Evolution of Patterning Mechanisms: Gleanings from Insect Embryogenesis and Spermatogenesis*. Cambridge University Press; 1983.
6. Duboule D: **Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony**. *Dev Suppl* 1994, **42**:135-142.
7. Richardson MK: **Heterochrony and the phylotypic period**. *Dev Biol* 1995, **172**:412-421.
8. Richardson MK, Hanken J, Gooneratne MJ, Pieau C, Raynaud A, Selwood L, Wright GM: **There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolutions and development**. *Anat Embryol* 1997, **196**:91-106.

9. Raff RA: *The Shape of Life: Genes, Development and the Evolution of Animal Form*. University Chicago Press; 1996.

10. Schleip W: *Die Determination der Primitiventwicklung*. Akademische Verlags-Gesellschaft; 1929.

11. Bininda-Emonds ORP, Jeffery JE, Richardson MK: **Inverting the hourglass: quantitative evidence against the phylotypic stage in vertebrate development**. *Proc Biol Sci* 2003, **270**:341-346.

12. Roux J, Robinson-Rechavi M: **Developmental constraints on vertebrate genome evolution**. *PLoS Genet* 2008, **4**:e1000311.

13. Comte A, Roux J, Robinson-Rechavi M: **Molecular signaling in zebrafish development and the vertebrate phylotypic period**. *Evol Dev* 2010, **12**:144-156.

14. Piasecka B, Lichocki P, Moretti S, Bergmann S, Robinson-Rechavi M: **The hourglass and the early conservation models-co-existing patterns of developmental constraints in vertebrates**. *PLoS Genet* 2013, **9**:e1003476.

15. Pantalacci S, Sémon M: **Transcriptomics of developing embryos and organs: a raising tool for evo-devo**. *J Exp Zool Part B Mol Dev Evol* 2015, **324**:363-371.

16. Roux J, Rosikiewicz M, Robinson-Rechavi M: **What to compare and how: comparative transcriptomics for evo-devo**. *J Exp Zool Part B Mol Dev Evol* 2015, **324**:372-382.

17. Domazet-Loo T, Tautz D: **A phylogenetically based transcriptome age index mirrors ontogenetic divergence patterns**. *Nature* 2010, **468**:815-818.

18. Kalinka AT, Varga KM, Gerrard DT, Preibisch S, Corcoran DL, Jarrells J, Ohler U, Bergman CM, Tomancak P: **Gene expression divergence recapitulates the developmental hourglass model**. *Nature* 2010, **468**:811-814.

19. Yanai I, Peshkin L, Jorgensen P, Kirschner MW: **Mapping gene expression in two xenopus species: evolutionary constraints and developmental flexibility**. *Dev Cell* 2011, **20**:483-496.

20. Irie N, Kuratani S: **Comparative transcriptome analysis reveals vertebrate phylotypic period during organogenesis**. *Nat Commun* 2011, **2**:248.

21. Levin M, Hashimshony T, Wagner F, Yanai I: **Developmental milestones punctuate gene expression in the *Caenorhabditis* embryo**. *Dev Cell* 2012, **22**:1101-1108.

22. Wang Z, Pascual-Anaya J, Zadissa A, Li W, Niimura Y, Huang Z, Li C, White S, Xiong Z, Fang D *et al.*: **The draft genomes of soft-shell turtle and green sea turtle yield insights into the development and evolution of the turtle-specific body plan**. *Nat Genet* 2013, **45**:701-706.

23. Gerstein MB, Rozowsky J, Yan K, Wang D, Cheng C, Brown JB, Davis CA, Hillier L, Sisu C, Li JJ *et al.*: **Comparative analysis of the transcriptome across distant species**. *Nature* 2014, **512**:445-448.

24. Drost H-G, Gabel A, Grosse I, Quint M: **Evidence for active maintenance of phylotranscriptomic hourglass patterns in animal and plant embryogenesis**. *Mol Biol Evol* 2015, **32**:1221-1231.

25. •• Levin M, Anavy L, Cole AG, Winter E, Mostov N, Khair S, Senderovich N, Kovalev E, Silver DH, Feder M *et al.*: **The mid-developmental transition and the evolution of animal body plans**. *Nature* 2016, **531**:637-641.

This comparison of developmental transcriptome series in ten species from ten different phyla aims to test the existence of phylotypic periods across phyla. The authors report that early and late development are linked by a 'mid-developmental transitions' relying on species-specific signalling pathways.

26. Xu F, Domazet-Loo T, Fan D, Dunwell TL, Li L, Fang X, Zhang G: **High expression of new genes in trochophore enlightening the ontogeny and evolution of trochozoans**. *Sci Rep* 2016, **6**:34664.

27. Wardlaw C: *Embryogenesis in Plants*. Methuen; 1955.

28. Pollock EG, Jensen WA: **Cell development during early embryogenesis in *Capsella* and *Gossypium***. *Am J Bot* 1964, **51**:915-921.

29. Mordhorst A, Toonen M, de Vries S, Meinke D: **Plant embryogenesis**. *CRC Crit Rev Plant Sci* 1997, **16**:535-576.

30. •• Cridge AG, Dearden PK, Brownfield LR: **Convergent occurrence of the developmental hourglass in plant and animal embryogenesis?** *Ann Bot* 2016, **117**:833-843.

In this review the authors summarize the literature on comparative morphology in plants, concluding that a developmental hourglass possibly limits morphological variation.

31. Raghavan V, Sharma K: *Zygotic Embryogenesis in Gymnosperms and Angiosperms*. Kluwer Academic Publishers; 1995.

32. Chandler J, Nardmann J, Werr W: **Plant development revolves around axes**. *Trends Plant Sci* 2008, **13**:78-84.

33. Quint M, Drost H-G, Gabel A, Ullrich KK, Bönn M, Grosse I: **A transcriptomic hourglass in plant embryogenesis**. *Nature* 2012, **490**:98-101.

34. Gossmann TI, Saleh D, Schmid MW, Spence MA, Schmid KJ: **Transcriptomes of plant gametophytes have a higher proportion of rapidly evolving and young genes than sporophytes**. *Mol Biol Evol* 2016, **33**:1669-1678.

35. • Cheng X, Hui JHL, Lee YY, Wan Law PT, Kwan HS: **A "Developmental Hourglass" in fungi**. *Mol Biol Evol* 2015, **32**:1556-1566.

The authors detect a transcriptomic hourglass in fungus sporocarp development. As fungi do not perform embryogenesis, this finding suggests that more fundamental processes than organogenesis are able to generate developmental hourglass patterns by constraining mid-developmental transitions.

36. Huijser P, Schmid M: **The control of developmental phase transitions in plants**. *Development* 2011, **138**:4117-4129.

37. •• Drost H-G, Bellstädt J, Ó'Maoiléidigh DS, Silva AT, Gabel A, Weinholdt C, Ryan PT, Dekkers BJW, Bentsink L, Hilhorst HWM *et al.*: **Post-embryonic hourglass patterns mark ontogenetic transitions in plant development**. *Mol Biol Evol* 2016, **33**:1158-1163.

This study finds that in plants post-embryonic developmental transitions, that do not include organogenic processes, are also associated with hourglass patterns.

38. Dekkers BJW, Pearce S, van Bolderen-Veldkamp RP, Marshall A, Widera P, Gilbert J, Drost H-G, Bassel GW, Müller K, King JR *et al.*: **Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination**. *Plant Physiol* 2013, **163**:205-215.

39. Silva AT, Ribone PA, Chan RL, Ligterink W, Hilhorst HWM: **A predictive coexpression network identifies novel genes controlling the seed-to-seedling phase transition in *Arabidopsis thaliana***. *Plant Physiol* 2016, **170**:2218-2231.